Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Claim 1. (Currently Amended) A [[M]]method of modeling ~~and/~~or obtaining tissue or tissue-like structures comprising

(a) culturing an embryonic stem (ES) cell-derived first cell type in the presence of at least one embryonic second cell type; and

(b) allowing integration and alignment of said at least two cell types into tissue or tissue-like structures.

Claim 2. (Original) The method of claim 1, wherein the ES cell of said ES cell-derived first cell type comprises a selectable marker operably linked to a first cell type-specific regulatory sequence specific for said first cell type.

Claim 3. (Original) The method of claim 2, wherein said selectable marker confers resistance to puromycin.

Claim 4. (Currently Amended) The method of ~~any one of claims 1 to 3~~ claim 1, wherein ~~said~~ the ES cell of said ES cell-derived first cell type comprises a reporter gene operably linked to a cell type-specific regulatory sequence specific for said first cell type.

Claim 5. (Original) The method of claim 4, wherein said cell type-specific regulatory sequence of the reporter gene is substantially the same as said first cell type-specific regulatory sequence of the marker gene.

Claim 6. (Original) The method of claim 5, wherein said reporter is selected from different color versions of enhanced green fluorescent protein (EGFP).

Claim 7. (Currently Amended) The method of ~~any one of claims 4 to 6~~ claim 5, wherein said marker gene and said reporter gene are contained ~~one~~ in the same recombinant nucleic acid molecule.

Claim 8. (Currently Amended) The method of claim 7, wherein said marker gene and said reporter gene are contained ~~on~~ in the same cistron.

Claim 9. (Currently Amended) The method of ~~any one of claims 1 to 8~~ claim 1, wherein said first cell type is selected from the group consisting of neuronal cells, glial cells, cardiomyocytes, glucose-responsive insulin-secreting pancreatic beta cells, hepatocytes, astrocytes, oligodendrocytes, chondrocytes, osteoblasts, retinal pigment epithelial cells, fibroblasts, keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, vascular endothelial cells, testicular progenitors, smooth and skeletal muscle cells.

Claim 10. (Currently Amended) The method of ~~any one of claims 1 to 9~~ claim 9, wherein said first cell type are cardiomyocytes.

Claim 11. (Currently Amended) The method of claim 10, wherein said first cell type-specific regulatory sequence is atrial- ~~and/~~or ventricular- specific.

Claim 12. (Currently Amended) The method of claim 10 ~~or 11~~, wherein said at least one embryonic second cell type are fibroblasts or endothelial cells.

Claim 13. (Currently Amended) The method of ~~any one of claims 1 to 12~~ claim 1, further comprising culturing said at least two cell types in the presence of an embryonic or embryonic stem (ES) cell-derived third cell type.

Claim 14. (Original) The method of claim 13, wherein said third cell type are endothelial cells or fibroblasts.

Claim 15. (Currently Amended) A co-culture of cells ~~as defined in any one of claims 1 to 14~~ obtainable by culturing the cells of claim 1.

Claim 16. (Currently Amended) A tissue obtainable by the method of ~~any one of claims 1 to 14~~ claim 1.

Claim 17. (Currently Amended) A method ~~of~~ for improving tissue repair or ~~and/~~organ function in a mammal comprising ~~the steps of~~:

(a) introducing a cellular inoculum comprising ~~a~~ said co-culture of cells of claim 15 in which differentiation has been initiated ~~or tissue of claim 16~~ to at least a portion of the previously damaged area of the tissue; and

(b) allowing said introduced cellular inoculum to engraft in situ as viable cells ~~or tissue~~ situated within the previously damaged area of ~~the~~ said tissue, wherein the engraftment results in improved tissue ~~and/~~or organ function or both in said mammal.

Claim 18. (Currently Amended) A method for improving cardiac function in a mammal after a myocardial infarct, said method comprising ~~the steps of~~:

(a) culturing undifferentiated mammalian embryonic stem (ES) cells comprising a resistance gene and a reporter gene under the control of the same cardiac-specific promoter in vitro in a culture medium containing the selective agent for the resistance gene under conditions allowing for differentiation of said ES cells into cardiomyocytes;

(b) isolating said differentiated cardiomyocytes ~~and/~~or eliminating non-differentiated cells or both, ~~optionally~~ along with cells differentiating towards irrelevant cell types from said cardiomyocytes in the course of differentiation;

(c) subsequently co-transplanting said cardiomyocytes with embryonic or ES cell-derived fibroblasts to at least a portion of the previously infarcted area of the heart tissue; and

(d) allowing said introduced cellular inoculum to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

Claim 19. (Currently Amended) The method of claim 18, wherein said resistance gene and said reporter gene are contained in a bicistronic vector and separated by an internal ribosome entry site (IRES).

Claim 20. (Original) The method of claim 19, wherein said resistance gene confers resistance to puromycin, said marker is EGFP and said promoter is the cardiac αMHC promoter.

Claim 21. (Currently Amended) A [[M]]method of modeling ~~and/~~or obtaining tissue or tissue-like structures comprising ~~the following steps~~:

(a) transfecting one or more multi- or pluripotent cells with recombinant nucleic acid molecules comprising a first cell type-specific regulatory sequence and a second cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said second cell type is different from said first cell type;

(b) culturing the cells under conditions allowing differentiation of ~~the~~ said cells; and

(c) isolating cells of at least two differentiated cell types ~~and/~~or eliminating non-differentiated cells, ~~optionally~~ along with cells differentiating towards irrelevant cell types from cell types of interest that activate the selectable marker in the course of differentiation.

Claim 22. (Currently Amended) The method of claim 21, further comprising transfecting said one or more cells with recombinant nucleic acid molecules comprising

at least one further cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said at least one further type is different from said first cell type and second cell type.

Claim 23. (Currently Amended) The method of claim 21 ~~or 22~~, wherein said cells are embryonic stem (ES) or embryonic germ (EG) cells.

Claim 24. (Currently Amended) The method of ~~any one of claims 21 to 23~~ claim 21, wherein said recombinant nucleic acid molecules are comprised in the same vector or different vectors.

Claim 25. (Currently Amended) The method of ~~any of claims 21 to 24~~ claim 21, wherein said cell type is selected from the group consisting of neuronal cells, glial cells, cardiomyocytes, glucose-responsive insulin-secreting pancreatic beta cells, hepatocytes, astrocytes, oligodendrocytes, chondrocytes, osteoblasts, retinal pigment epithelial cells, fibroblasts, keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, vascular endothelial cells, testicular progenitors, smooth and skeletal muscle cells.

Claim 26. (Currently Amended) The method of claim ~~7 or 8~~ 18, wherein said promoter is selected from the group consisting of αMHC, MLC2V, catherin, Tie-2 and collagen promoter.

Claim 27. (Currently Amended) The method of ~~any one of claims 21 to 26~~ claim 21, wherein said one or more recombinant nucleic acid molecules are transfected concomitantly or subsequently into said one or more cells.

Claim 28. (Currently Amended) The method of ~~any one of claims 21 to 26~~ claim 21, wherein at least two different cells or clones thereof are transfected and selected, wherein said at least two different cells or cell clones thereof contain recombinant nucleic acid molecules with different cell type-specific regulatory sequences.

Claim 29. (Currently Amended) The method of claim 28, wherein said at least two different cells or cell clones thereof are mixed at the initial stage of differentiation in order to allow formation of cell aggregates.

Claim 30. (Original) The method of claim 29, wherein said cell aggregates are chimeric embryoid bodies (EBs).

Claim 31. (Currently Amended) The method of ~~any one of claims 21 to 30~~ claim 21, wherein one of said cells or cell clones thereof is transfected and selected, wherein said cell or cell clones thereof contains recombinant nucleic acid molecules with at least two different cell type-specific regulatory sequences.

Claim 32. (Currently Amended) The method of ~~any one of claims 21 to 31~~ claim 21, wherein at least two of said selectable marker operably linked to said different cell type-specific regulatory sequences are identical.

Claim 33. (Currently Amended) The method of ~~any one of claims 21 to 32~~ claim 21, wherein at least one of said selectable marker is operably linked to said different cell type-specific regulatory sequences confers resistance to puromycin, bleomycin, hygromycin, methothrexate, or neomycin.

Claim 34. (Currently Amended) The method of ~~any one of claims 21 to 33~~ claim 21, wherein one or more of said recombinant nucleic acid molecules further comprise a reporter operably linked to said cell type-specific sequence.

Claim 35. (Original) The method of claim 34, wherein said reporter is selected from different color versions of enhanced green fluorescent protein (EGFP).

Claim 36. (Currently Amended) The method of claim 35, wherein EYFP (yellow), ECFP (blue) ~~and/~~or hcRFP (red) are operably linked to different cell type-specific sequences.

Claim 37. (Currently Amended) The method of ~~any one of claims 34 to 36~~ claim 34, wherein said selectable marker and said reporter are ~~expressed from~~ on a bicistronic vector.

Claim 38. (Original) The method of claim 37, further comprising one or more internal ribosomal entry sites (IRES), wherein said IRES separates said selectable marker and said reporter.

Claim 39. (Currently Amended) The method of ~~any one of claims 21 to 38~~ claim 21, further comprising allowing self-assembly of the different cell types.

Claim 40. (Currently Amended) The method of any one of claims 1 ~~to 14~~ or 21 ~~to 39~~, further comprising ~~analysing~~ analyzing the physiological ~~and/~~or developmental status or both of the cells or cell aggregate.

Claim 41. (Original) The method of claim 40, wherein the status is analyzed by monitoring the differentiation of electrical activity of the cells on an array.

Claim 42. (Original) The method of claim 41, wherein said status is analyzed by recording the extracellular field potentials with a microelectrode array (MEA).

Claim 43. (Currently Amended) A cell or cells obtainable by the method of ~~any one of claims 21 to 42~~ claim 21, wherein said cell or cells are capable of differentiating into at least two cell types.

Claim 44. (Currently Amended) A cell aggregate of at least two different cell types obtainable by the method of ~~any one of claims 21 to 42~~ claim 21.

Claim 45. (Currently Amended) A tissue obtainable by the method of any one of claims ~~1 to 42 or comprising cells of claim 43 or a cell aggregate of claim 44~~ 1 or 21.

Claim 46. (Currently Amended) An organ comprising cells of claim 43~~, a cell aggregate of claim 44 or tissue of claim 45~~.

Claim 47. (Currently Amended) An implant or transplant comprising cells of claim 43~~, a cell aggregate of claim 44, a tissue of claim 45, or an organ of claim 46~~.

Claim 48. (Currently Amended) A composition of matter comprising recombinant nucleic acid molecules ~~as defined in any one of claims 21 to 42, cells of claim 43, a cell aggregate of claim 44, or a tissue of claim 45~~ of claim 21.

Claim 49. (Currently Amended) ~~Use of the~~ The method of any one of claims 1 ~~to 14~~ or 21 ~~to 42~~ for analyzing early steps of tissue formation during embryonic development or the influence of factors and compounds on this process.

Claim 50. (Currently Amended) A method of treatment of damaged tissue or organs in a subject comprising implanting or transplanting to the subject in need thereof cells of claim 43~~, a cell aggregate of claim 44, a tissue of claim 45 or an organ of claim 46~~.

Claim 51. (Currently Amended) A method for improving cardiac function in a mammal after a myocardial infarct, said method comprising ~~the steps of~~:

(a) transfecting mammalian embryonic stem (ES) cells with a recombinant nucleic acid molecule comprising a resistance gene under the control of cardiac, fibroblast ~~and/~~or endothelium-specific regulatory sequences, and ~~optionally~~ comprising one or more reporters under the same specific regulatory sequences;

(b) culturing said ES cells in vitro in a culture medium containing the selective agent for the resistance gene under conditions allowing differentiation of said ES cells into cardiomyocytes, fibroblasts ~~and/~~or endothelial cells;

(c) eliminating from said differentiated cardiomyocytes, fibroblasts ~~and/~~or endothelial cells non-differentiated cells, ~~optionally~~ along with cells differentiating towards irrelevant cell types; ~~optionally~~

(d) allowing aligning and integration of said differentiating cardiomyocytes, said fibroblasts ~~and/~~or said endothelial cells into cardiac-like tissue; and

(e) subsequently co-transplanting said cardiomyocytes, said fibroblasts ~~and/~~or said endothelial cells or said tissue to at least a portion of the previously infarcted area of the heart tissue; and

(f) allowing said introduced cells or tissue to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

Claim 52. (Currently Amended) The method of claim 51, wherein said cardiomyocytes, fibroblasts ~~and/~~or endothelial cells are ~~derived~~ obtainable from the same ES cell.

Claim 53. (Currently Amended) The method of claim 51, wherein said cardiomyocytes, fibroblasts ~~and/~~or endothelial cells are ~~derived~~ obtainable from different ES cells.

Claim 54. (Currently Amended) The method of ~~any of claims 51 to 53~~ claim 51, wherein said cardiac-specific regulatory sequence is selected from the group ~~promoters~~ consisting of αMHC, MLC22v, MLC1a, MLC2a and βMHC, wherein said fibroblast-specific regulatory sequence is selected from the group ~~promoters~~ consisting of Tie2, Tie1 and Catherin, and wherein said endothelium-specific regulatory sequence is selected from the group ~~promoters~~ consisting of collagen I promoters.

Claim 55. (Currently Amended) The method of ~~any of claims 51 to 54~~ claim 54, wherein said reporter for said cardiomyocytes, fibroblasts ~~and/~~or endothelial cells is independently selected from the enhanced green fluorescent proteins ECFP (blue), EYFP (yellow) and hcRFP (red).

Claim 56. (Currently Amended) The method of ~~any of claims 51 to 55~~ claim 51, wherein said resistance gene and said reporter are separated by an internal ribosomal entry site (IRES).

Claim 57. (Currently Amended) A vector or a composition of vectors comprising ~~the~~ said recombinant nucleic acid molecule[[s]] ~~as defined in any one of claims 51 to 56~~ of claim 51.

Claim 58. (Currently Amended) A cell or a plurality of cells comprising ~~the~~ said vector or ~~the~~ said composition of vectors of claim 57.

Claim 59. (Currently Amended) An array comprising a solid support and attached thereto or suspended thereon cells of claim 43 or 58~~, a cell aggregate of claim 44, or a tissue of claim 45~~.

Claim 60. (Currently Amended) The array of claim 59, ~~which~~ wherein said array is a microelectrode array (MEA).

Claim 61. (Currently Amended) An apparatus for analyzing ~~the~~ said array of claim 59~~ or 60~~.

Claim 62. (Currently Amended) A method for obtaining ~~and/~~or profiling a test substance capable of influencing cell development ~~and/~~or tissue structure formation or both comprising~~ the steps~~:

(a) contacting a test sample comprising cells of claim 43 or 58~~, a cell aggregate of claim 44, a tissue of claim 45, an organ of claim 46 or an array of claim 59 or 60~~ with a test substance; and

(b) determining a phenotypic response in said test sample compared to a control sample, wherein a change in the phenotypic response in said test sample compared to the control sample is an indication that said test substance has an effect on cell development ~~and/~~or tissue structure formation or both.

Claim 63. (Currently Amended) The method of claim 62, wherein said test sample is contacted with said test substance prior to, during or after said cell or cell aggregate has passed through the method of any one of claims 1~~ to 14~~ or 21~~ to 42~~.

Claim 64. (Currently Amended) The method of claim 62 ~~or 63~~, wherein said contacting step further includes contacting said test sample with at least one second test substance in the presence of said first test substance.

Claim 65. (Currently Amended) The method of ~~any one of claims 62 to 64~~ claim 62, wherein preferably in a first screen said test substance is comprised in and subjected as a collection of test substances.

Claim 66. (Original) The method of claim 65, wherein said collection of test substances has a diversity of about 10^3 to about 10^5.

Claim 67. (Original) The method of claim 66, wherein the diversity of said collection of test substances is successively reduced.

Claim 68. (Currently Amended) The method of ~~any one of claims 61 to 67~~ claim 62, which is performed on an array ~~as defined in claim 59 or 60~~.

Claim 69. (Currently Amended) The method of ~~any one of claims 61 to 68~~ claim 62, wherein ~~the~~ said phenotypic response comprises electrophysiological properties during the ongoing differentiation process.

Claim 70. (Currently Amended) The method of any one of claims 1 ~~to 14, 21 to 42 or 62 to 69~~ or 21, wherein said one or more cells are genetically engineered to (over)express or inhibit the expression of a target gene.

Claim 71. (Currently Amended) The method of any one of claims 1~~ to 14, 21 to 42 or 62 to 70~~ or 21, wherein a compound known to activate or inhibit differentiation process ~~and/~~or tissue structure formation or both is added to the culture medium.

Claim 72. (Currently Amended) The method of any one of claims 1~~ to 14, 21 to 42 or 62 to 71~~ or 21, wherein said one or more cells or tissue are contained in a container.

Claim 73. (Currently Amended) The method of any one of claims 1~~ to 14, 21 to 42 or 62 to 72~~ or 21, comprising taking ~~3~~ three or more measurements, optionally at different positions within the container.

Claim 74 (Currently Amended) The method of ~~any one of claims 72 or 73~~ claim 72, wherein said container is a well in a microtiter plate.

Claim 75. (Original) The method of claim 74, wherein said microtiter plate is a 24-, 96-, 384- or 1586- well plate.

Claim 76. (Currently Amended) A method of manufacturing a drug comprising the steps of ~~any one of claims 62 to 75~~ claim 62.

Claim 77. (Currently Amended) A method of manufacturing an agent which supports wound healing ~~and/~~or healing of damaged tissue or both comprising ~~the steps of any one of claims 62 to 76~~ the method of claim 62.

Claim 78. (Currently Amended) The method of claim 76 ~~or 77~~, further comprising modifying said test substance to alter, eliminate ~~and/~~or derivatize a portion ~~thereof~~ of said test substance suspected of causing toxicity, increasing bioavailability, solubility ~~and/~~or half-life or any combination thereof.

Claim 79. (Currently Amended) The method of ~~any one of claims 76 to 78~~ claim 78, further comprising mixing ~~the~~ said test substance isolated or modified with a pharmaceutically acceptable carrier.

Claim 80. (Currently Amended) A kit or composition useful for conducting [[a]] the method of any one of claims 1 ~~to 14~~, 21 ~~to 42~~, ~~50 to 56~~ or 51 comprising ~~or 62 to 79, containing~~ the vector or the composition of vectors of claim 57, a multi- or pluripotent cell, and ~~optionally~~ culture medium, recombinant nucleic acid molecules, or standard compounds.

Claim 81. Cancelled.